EXHIBIT A

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In Vivo Caries-Like Lesion Prevention with Argon Laser: Pilot Study

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ABSTRACT

Objective: **This clinical pilot study was conducted to investigate the effectiveness of argon laser irradiation to reduce demineralization or loss of tooth structure *in vivo*. *Summary Background Data: In vitro* research previously demonstrated the ability of argon laser irradiation to reduce demineralization or loss of tooth structure. *Methods:* Using the Ogaard model of producing demineralization, the experimental teeth were irradiated with argon laser of 250 mW (producing approximately 12 J/cm²) prior to banding. Polarized light evaluation of the sectioned, extracted teeth was used to determine the amount of demineralization. *Results:* Results showed a 29.1% reduction in demineralization in the experimental teeth as compared to the bilateral control teeth. *Conclusion:* Low-power argon laser irradiation significantly reduced demineralization clinically.**

INTRODUCTION

Previous studies have recorded the reduction of caries-like lesions or demineralization of extracted human teeth that have been irradiated with CO_2 laser energies. Stern et. al.,[1] in 1972, described the reduction of subsurface lesions after CO_2 laser irradiation. Since then, others have reported that the CO_2 laser of the appropriate wavelength reduced demineralization in enamel,[2,3] showed a slowing of enamel initial solution rates,[4] reduced occlusal surface demineralization,[5] and was effective in both pulsed and continuous wave mode.[6] The addition of a chemical inhibitor to the lasing procedure and/or controlling the wavelength and power resulted in even greater reduction in caries-like lesions.[7–9] The argon laser has also been reported to be effective in reducing loss of tooth structure or size of subsurface lesions on both enamel and root surfaces.[10–12] More recent studies demonstrated that combined with fluoride surface treatment or in the demineralization solution, even further reduction in loss of tooth structure or size of lesion occurred.[13–15]

Dr. John Featherstone describes demineralization caused by acid attack as the precursor to dental caries.[16] When sufficient mineral has been removed from the enamel, the structure collapses and cavity formation (dental caries) results that can be seen visually or by radiograph. Therefore, if we can prevent demineralization, we can reduce or prevent dental caries (tooth decay).

This *in vivo* study was conducted to evaluate the clinical effectiveness of the argon laser to prevent or reduce demineralization of enamel and thereby prevent or reduce caries formation.

MATERIALS AND METHODS

Bicuspids scheduled for orthodontic extraction and utilization of a modified orthodontic band as described by Ogaard and Rolla[17] were used to develop demineralization of human enamel *in vivo*. The protocol was approved by the Institutional Review Board (IRB) of Creighton University.

Four orthodontic patients scheduled for extraction of bilateral bicuspids participated in the pilot study. After initial screening and health history, the patients had full mouth impressions taken and study models made. After the visit, preformed orthodontic bands were fitted to the models, and modified by welding two metal posts to the inner surface of the band to create a crevice between the band and buccal surface of the tooth for plaque accumulation (Fig. 1). At the second appointment (approximately 1 week later), the experimental tooth was

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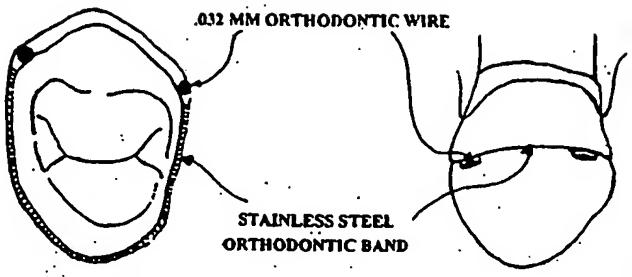

FIG. 1. Creation of a crevice for plaque accumulation.

lased with argon laser irradiation, approximately 12 J/cm² by an HGM Model 5 argon laser using a 250-mW, approximately a 5-mm diameter beam for 10 seconds. The control tooth on the opposite side of the mouth was not lased. The modified orthodontic bands were then cemented in place on both the experimental and the control tooth.

Five weeks after the time of band placement, the teeth were extracted by an oral surgeon at Creighton University. After extraction and removal of the band, the teeth (both control and experimental) were sectioned longitudinally through the demineralization lesions and prepared for polarized light evaluation. The sections were imbibed with water and photomicrographs were taken. Mean surface zone and body of the lesion depths were obtained using a Visilog 5.1.1 image analysis software (Noesis Vision Inc., Quebec, Canada) and taking measurements along the inner aspect of the surface zone and body of the lesion. In the [Because of the] research design, the body of the lesion depths from the bilateral lased and control teeth from the same patient were compared providing the statistical difference in demineralization for that patient.

RESULTS

The argon laser irradiation significantly reduced the demineralization of the enamel under the experimental conditions. The teeth that were lased prior to band placement averaged a 29.1% reduction in lesion depth as compared to the unlased teeth (Table 1). The reduction ranged from 23.4% for pair #4, to 33% in pair. Measurements were taken from the photomicrographs of the teeth (Figs. 2, 3).

The reduction in depth of demineralization (lesion depth) was statistically significant ($p < 0.05$) for the lased samples as compared to the unlased controls.

TABLE 1. DEMINERALIZATION OF BILATERAL PAIRED BICUSPIDS

		Depth of Lesions (mm)	
Pair 1	Control tooth	.17959	
	Lased tooth	.11976	
		.05983	33% decrease
Pair 2	Control tooth	.313622	
	Lased tooth	.213445	
		.100177	31.9% decrease
Pair 3	Control tooth	.178528	
	Lased tooth	.128218	
		.50310	28.1% decrease
Pair 4	Control tooth	.154163	
	Lased tooth	.118004	
		.36159	23.4% decrease
Range			23.4%–33%
Average decrease			29.1%

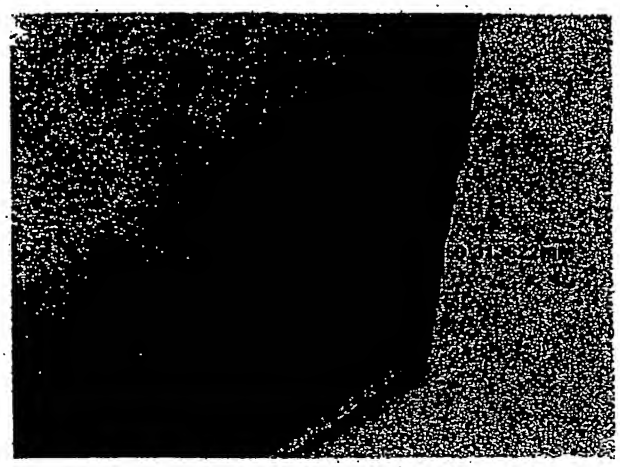

FIG. 2. Control group (no treatment) at 5 weeks.

DISCUSSION

Although the effects of laser irradiation on dental caries and tooth structure were explored some 30 years ago,[18–20] the risk of thermal damage to the adjacent hard tissue and pulp was such that much of the research was abandoned. However, with improved laser technology, a number of different types of lasers with varying tissue penetration and energy levels have been developed.[2] Currently, a number of lasers that emit different wavelengths and varied energy levels have been adapted for use

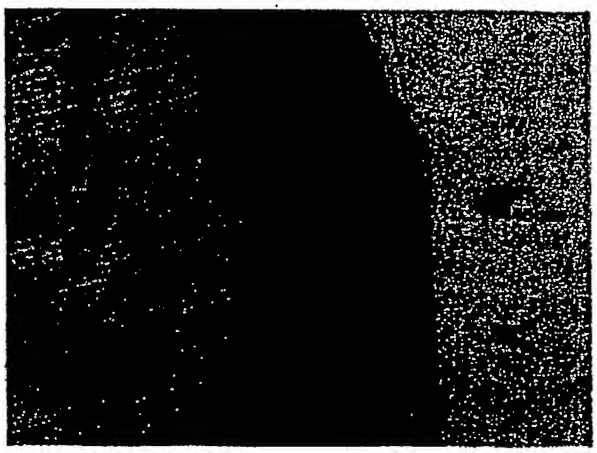

FIG. 3. Experimental group treated with argon laser for 10 seconds at 250 mW at 5 weeks.

in dentistry.[22] One of these is the argon laser (emitting primarily 476, 488, and 514 nm) currently cleared by the Food and Drug Administration (FDA) for clinical use in photopolymerization of light-activated materials, oral soft tissue surgery, bleaching of teeth, and is used clinically by practitioners as an adjunct to periodontal therapy, for pulpotomies and root canal therapy. Several reports have been published on the *in vitro* success of the argon laser to reduce the loss of tooth structure or demineralization[10–15] but not *in vivo*. This study is the first *in vivo* report of successfully reducing the amount of demineralization clinically with the argon laser or any laser in the United States. The argon laser was chosen for use because its success *in vitro* in reducing demineralization, its safety of use has been established by research, and it has been cleared [clearance] by the FDA for these powers on tooth structure for other purposes.

The Ogaard and Rolla model[17] was chosen because of its ability to develop demineralization in a short period of time at no risk to the patient because the tooth was scheduled for extraction.

CONCLUSION

These first clinical results are consistent with laboratory results that low power laser irradiation (250 mW, approximately 12 J/cm^2) can significantly reduce demineralization of enamel.

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